CA 15-3-CHECK-1

Quantitative determination of Cancer Antigen 15-3 (CA 15-3) in whole blood, plasma or serum samples

FOR EASY READER® OR EASY READER+® USE ONLY

Ref.: 68091 (20 tests) & Ref.: 68091-10T (10 tests)

I- PRINCIPLE

Breast cancer is the most common life-threatening malignant lesion in women of many developed countries today (1).

Around half of these newly diagnosed patients are node-negative, however 30% of these cases progress to metastatic disease. There are a number of tumor markers that can help to identify and diagnose which breast cancers will be an aggressive disease or which ones will have an indolent cause (2,3, 4).

Among them, CA 15-3, which is an epitope of the protein antigen encoded by the breast cancer MUC1 gene, is the most frequently used marker in the management of invasive metastatic breast cancer (5).

It correlates well with the extend of disseminated mammary carcinoma (6) and with therapeutic response to evaluate the disease progression or recurrence (7).

The CA 15-3-CHECK-1 test is a rapid quantitative assay for the detection of CA 15-3 in serum, plasma or whole blood. The method employs a unique combination of monoclonal-dye conjugate and polyclonal solid phase antibodies to identify CA 15-3 in the test samples with a high degree of specificity.

As the test sample flows through the absorbent device, the labelled antibody-dye conjugate binds to the CA 15-3 forming an antibody-antigen complex. This complex binds to the anti-CA 15-3 antibody in the positive reaction zone (T) and produces a pink-rose colour band.

In the absence of CA 15-3, there is no line in the positive reaction zone (T). The reaction mixture continues flowing through the absorbent device past the reaction zone (T) and control zone (C).

Depending on the CA 15-3 concentration level, different lines of different intensities will appear on the reading window allowing the quantitative measurement of CA 15-3 when used in combination with the VEDALAB's rapid test readers.

II- CA 15-3-CHECK-1 KIT COMPONENTS

Each kit contains everything needed to perform 10 or 20 tests.

1- CA 15-3- CHECK-1 reaction devices:	10	20
2- Disposable plastic pipettes:	10	20
3- Diluent in a dropper bottle:	2.5 mL	5 mL
4- Instruction leaflet:	1	1

5- Controls (Optional):

Positive control (ref. V6800) and Negative control (ref. V6801): a freeze-dried preparation of a non-infectious compound in diluted human serum, tested and found negative for anti-HIV, anti-HCV and HBs antigen, containing 0.05 % sodium azide is optionally available as a positive and negative control (1x 0.25 mL). The concentration range is indicated on the vial label.

III- STORAGE AND STABILITY

1- All CA 15-3-CHECK-1 kit components including optional controls before reconstitution should be stored at room temperature (+4°C to +30°C) in the sealed pouch.

2- ***Do not freeze the kit***.

3- The CA 15-3-CHECK-1 kit is stable until the expiry date stated on the package label.

IV- PRECAUTIONS

1- This test is designed for *in vitro* diagnostic use and professional use only.

2- Read carefully the instructions before using this test.

3- Handle all specimens as if they contained infectious agents. When the assay procedure is completed, dispose of specimens carefully after autoclaving them for at least one hour. Alternatively, they can be treated with 0.5% to 1% solution of sodium hypochlorite for one hour before disposal.

4- Wear protective clothing such as laboratory coats and disposable gloves while assaying samples.

5- Do not eat, drink or smoke in the area where specimens and kit reagents are handled.

6- Avoid any contact between hands and eyes or nose during specimen collection and testing.

7- Do not use beyond the expiry date which appears on the package label.

8- Do not use a test from a damaged protective wrapper.

V- SPECIMEN COLLECTION AND PREPARATION

1- CA 15-3-CHECK-1 test is to be performed on human serum, plasma or whole blood.

2- The specimen should be collected under the standard laboratory conditions (aseptically in such a way as to avoid haemolysis).

3- If anticoagulant is needed, only citrate, EDTA or heparin should be used.

4- Each specimen should be treated as if potentially infectious.

5- Whole blood samples should be tested immediately (< 4 hours). Finger prick samples should be assayed just after collection.

6- If the test is to be run within 48 hours after collection the specimen should be stored in the refrigerator (+2°C to +8°C). If testing is delayed more than 48 hours, the specimen should be frozen. The frozen specimen must be completely thawed, thoroughly mixed and brought to room temperature prior to testing. Avoid repeated freezing and thawing.

7- In case of cloudiness, high viscosity or presence of particulate matter into the serum specimen, it should be diluted with equal volume (V/V) of negative specimen before testing.

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VI- ASSAY PROCEDURE

<u>IMPORTANT</u>: Switch the reader on and allow it to warm up for at least 30 minutes before performing any measurements.

a) Controls testing

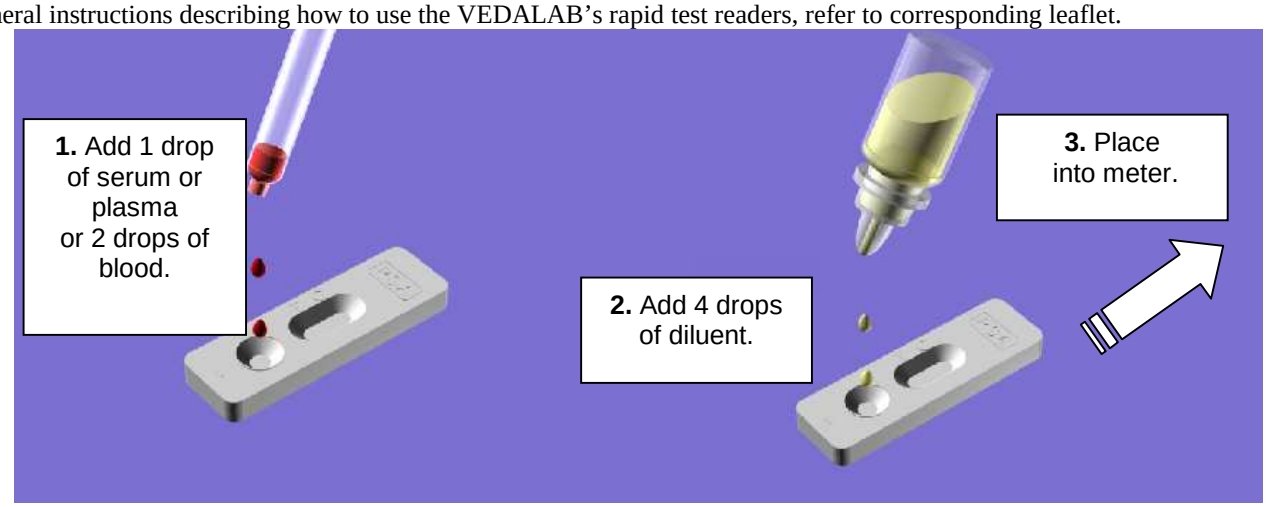
- Wait for 15 minutes after the freeze-dried control has been reconstituted and mix well few seconds (Vortex).
- Add the requested volume (25µL) with **<u>lab pipette (disposable tips)</u>** into the sample well of the cassette and proceed in the same way as for a patient's sample.
- The expected concentration level (**in IU/mL**) is indicated on the vial label and obtained result must match the indicated value. The concentration level can change slightly depending on lot number.
- **The reconstituted vial should be kept at +2°C to +8°C and should be used within 7 days after reconstitution**.

b) Samples testing

<u>Follow the below instructions or refer to the picture n°1.</u>

1- Allow samples and CA 15-3-CHECK-1 test devices to come to room temperature prior to testing.
2- Remove the reaction device from its protective wrapper by tearing along the split.
3- Label device with the patient's name or control number.
4- Fill the serum dropper with specimens (serum or plasma) and by holding it vertically, dispense one drop (25 µL) into sample well. If the whole blood is used, dispense two drops (50 µL) into the sample well (▷) **<u>and wait for the blood sample to be completely absorbed before adding diluent</u>**.
5- Hold the diluent vial vertically and slowly add exactly 4 drops of diluent (150 µL) in the sample well (▷) **<u>with an interval of 2-3 seconds between each drop.</u>**
6- Read the result **(in IU/mL**) after 10 minutes either using the immediate or countdown reading mode (see corresponding leaflet).

For general instructions describing how to use the VEDALAB's rapid test readers, refer to corresponding leaflet.



Picture n°1

VII- PERFORMANCES CHARACTERISTICS

a) Linearity

The measuring range is 5 – 200 IU/mL.
For CA 15-3 concentration below 5 IU/mL, the result will be given as "< 5 IU/mL".
For CA 15-3 concentration over 200 IU/mL, the result will be given as "> 200 IU/mL".
For samples whose concentration is higher than 200 IU/mL, dilute with saline and repeat the assay as per instructions of Part. VI. In that case, **the obtained concentration must be multiplied by the dilution factor in order to get the right value: for example, the concentration value obtained from a 10 times (1 volume of sample + 9 volumes of saline) diluted sample must be multiplied by 10 to get the sample CA 15-3 level before dilution.**

b) Accuracy

A study has been performed using serum samples obtained from dilutions of CA15-3 W.H.O. internal reference. Covering a range of 0 to 200 IU/mL, optical densities expressed as a function of CA15-3 concentrations are described by following polynomial curve:

$$Y = 10.10 + 3.81x - 0.01x^2 \quad (r = 0.987)$$

The results show a good correlation ($r > 0.98$) of the values obtained with CA 15-3-CHECK-1 on VEDALAB's readers.

c) Sensitivity

Concentrations close to 2.5 IU/mL are detected by CA 15-3-CHECK-1 test. In these cases, results will be rendered as "<5 IU/mL".
Levels higher than 35 IU/mL are generally considered as abnormal values.

d) Precision
A comparative study was performed using a panel of 18 human sera preassayed either on Roche Cobas ECLIA® or Biomerieux Mini-Vidas analysers and another panel of 26 human sera preassayed on Biomerieux Mini-Vidas analyser. Results showed an overall coefficient of correlation of 95.9% (CI 95% [93-98])* between CA 15-3-CHECK-1 quantitative rapid tests and EIA methods.
**CI 95% : 95% confidence interval.*

e) Intra-assay reproducibility
Within run precision was evaluated using 25 replicates of 3 samples containing 20, 80 and 160 IU/mL of CA 15-3 by serial dilutions of a preassayed serum sample.
The obtained CVs (coefficient of variation) were respectively equal to 17, 14.2 and 9.7%.

f) Inter-assay reproducibility
Inter lot reproducibility was evaluated using three serum samples containing 26.4, 54 and 78.1 ng/mL of CA 15-3 respectively and using 3 lots of CA 15-3-CHECK-1. The obtained CVs (coefficient of variation) are 11%, 6.7% and 3.6% respectively.

g) Hook effect
No hook effect was observed up to an CA 15-3 concentration of 5,000 IU/mL.

h) Expected values
Healthy women are expected to have CA 15-3 values below 35 IU/mL.

i) Interferences
1. Bilirubin: negative and positive spiked serum samples with 0.3 g/L of bilirubin showed repeatedly negative, weak and strong positive results.
2. Haemoglobin: negative and positive spiked serum samples with 5 g/L of haemoglobin showed repeatedly negative, weak and strong positive results.
3. Triglycerides: negative and positive spiked serum samples with 15 g/L of triglycerides showed repeatedly negative, weak and strong positive results.

VIII- LIMITATIONS
1- As for any diagnostic procedure, the physician should evaluate data obtained by the use of this test in light of other clinical information.
2- Some serum specimens with high rheumatoid factor (RF) concentration may yield non-specific positive results during testing. Such cases should be identified before testing.
3- The test is designed to eliminate the potential interference of human antibodies to murine IgG (HAMA). However high level of HAMA could give falsely positive results.
4- **Use only fresh whole blood samples (< 4 hours) when test is performed with blood samples. Finger prick samples should be assayed just after collection.**
5- Samples demonstrating gross haemolysis or turbidimetry should not be used with this test.
6- This format of test is to be only used with VEDALAB rapid test readers.
7- If the reading time (10 minutes) is not strictly respected, wrong results will be obtained.
8- This format of test should not be used for visual reading.
9- As it is true for any diagnostic method or for any measurements through analysers, there is a variability of the obtained result. Therefore, a confidence range of +/- 25% should be considered for the final value and for the clinical significance of the result.
10-Do not use the reader for measurements before at least 30 minutes warm-up after having switched on.

IX- BIBLIOGRAPHY

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3- **Kute TE, Shao ZM, Snugg NK, Long RT, Russell GB, Case LD.** Cathepsin D as a prognostic indicator for node-negative breast cancer patients using both immunoassays and enzymatic assays. Cancer Res. 1992; 52-198-203.
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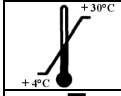
5- **Tonodini C. Hayes DF, Kufe D.** Circulating tumor markers in breast cancer. In: Henderson IC. ed. Diagnosis and therapy of breast cancer. Philadelphia: W.B Saunders, 1989: 653-74.
6- **Dnistran AM, Schwarz MK, Greenburg EJ, Smith CA, Schwarz DC.** CA 15-3, carcinoembryonic antigen in the clinical evaluation of breast cancer. Clin. Chem. Acta 1991, 200: 81-94.
7- **Bombadieri E., Pizzichetta M, Veronisi P. Seregni E., Bogni A, Maffioli L, et al.** CA 15-3 determination in patients with breast cancer. Eur. J. Cancer 1993; 29A: 144-6.

	Read the instructions before use		For *in vitro* diagnostic use
	Temperature limitations		Do not reuse
	Manufacturer		

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